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(71) Applicant *(for all designated States except US)*: COR THERAPEUTICS, INC. [US/US]; 256 E. Grand Avenue, South San Francisco, CA 94080 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: TOMLINSON, James, A. [US/US]; 1540 Balboa Street, Burlingame, CA 94010 (US).

(74) Agents: ADLER, Reid, G. et al.; Morgan, Lewis & Bockius LLP, 1800 M Street, N.W., Washington, DC 20036-5869 (US).

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(54) Title: CLONING AND CHARACTERIZATION OF A HUMAN ADENYLYL CYCLASE

(57) Abstract

A DNA sequence encoding a human adenylyl cyclase is described. The amino acid sequence of the adenylyl cyclase is also described.

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CCCGACCCCC CACCCCCCAC CCCCCCCCCC ACCCCCACCA CCCCCCCCCT   50
CCCCTCCCCC CTCCCCCCTC CCCCCCCCCC TCCCCCTCCT CCCCCCCCCA

CCCACCACCC CCCCACCCCC CCCCCCCCCC CCCCACCCCC CCCCCCCCCC  100
CCCTCCTCCC CCCCTCCCCC CCCCCCCCCC CCCCTCCCCC CCCCCCCCCC

CCCCCCCCCC CCCACCCCCA CCCCCCACCC CCCCCCCCCA CACCTCTCCA  150
CCCCCCCCCC CCCTCCCCTC CCCCCCTCCC CCCCCCCCTC CTTCTCCACT
                                                  MetSer

TCCTTTACTC CCCTCCTCCT CCCTAAACTC CACTACCCCA AACACCCCTC  200
ACCAATCCAC CCCACCACCA CCCATTTCAC CTACTTCCCT TTTCTCCCAC
TrpPheSerG lyLeuLeuVa lProLysVal AspGluArgL ysThrAlaTr

CCCTCAACCC AACTCCCCAA ACCCTTCCCC CCCCCTCCCC ACTCCCCCAC  250
CCCACTTCCC TTACCCCTCT TCCCACCCCC CCCCCCACCC TCACCCCCTC
pGlyGluArg AsnGlyGlnL ysArgSerAr gArgArgGly ThrArgAlaG

CTCCCTTCTC CACCCCCCCC TATCTCAACT CCCTCCCCCA TCCACACCCA  300
CACCCAACAC CTCCCCCCCC ATACTACTCA CCCACCCCCT ACCTCTCCCT
lyGlyPheCy sThrProArg TyrMetSerC ysLeuArgAs pAlaGluPro

CCCACCCCCA CCCCTCCCCC CCCCCCTCCC TCCCCCTCCC ACCATCACCC  350
CCCTCCCCCT CCCCACCCCC CCCCCCCACC ACCCCCACCC TCCTACTCCC
ProSerProT hrProAlaGl yProProArg CysProTrpG lnAspAspAl

CTTCATCCCC ACCCCCCCCC CACCCAACCC CAACCACCTC CCCCTCCCCC  400
CAACTACCCC CCCCACCCCC CTCCCTTCCC CTTCCTCCAC CCCCACCCCC
aPheIleArg ArgGlyGlyP roGlyLysGl yLysGluLeu GlyLeuArgA

CACTCCCCCT CCCCTTCCAC CATACCCACC TCACAACCAC ACCCCCCCCC  450
CTCACCCCCA CCCCAACCTC CTACTCCCTC ACTCTTCCTC TCCCCCCCCC
laValAlaLe uGlyPheGlu AspThrGlnV alThrThrTh rAlaGlyGly

ACCCCTCACC TCCCCCCCCA CCCCCTCCCC ACCACTCCCA CTTCCTCCTC  500
TCCCACTCCC ACCCCCCCCT CCCCCACCCC TCCTCACCCT CCACCACCAC
ThrAlaGluV alAlaProAs pAlaValPro ArgSerGlyA rgSerCysTr

CCCCCCTCTC CTCCACCCTC TCCACTCCAA CCACTTCCCT TCCCCCACCC  550
CCCCCCACAC CACCTCCACA ACCTCACCTT CCTCACCCCA ACCCCCTTCC
pArgArgLeu ValGlnValP heGlnSerLy sGlnPheArg SerAlaLysL

TCCACCACCT CTACCACCCC TACTTCTTCC ACATCACCCA CACCACCCTC  600
ACCTCCTCCA CATCCTCCCC ATCACACACC TCTACTTCCT CTCCTCCCAC
euGluHisLe uTyrGlnArg TyrPhePheG lnMetAsnGl nSerSerLeu
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Cloning and characterization of a human adenylyl cyclase

Field of the Invention

This invention relates to DNA encoding a human adenylyl cyclase. This invention also relates to the adenylyl cyclase encoded by that DNA. Referred to herein as the human type VI adenylyl cyclase (hAC6) polypeptide, this enzyme can be used as a tool to screen for agonists and antagonists that can either stimulate or inhibit type VI adenylyl cyclase activity. Such compounds have therapeutic utility in treating (1) diseases that are caused by aberrant activity of this enzyme and (2) diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of type VI adenylyl cyclase.

The present invention also relates to the isolated entire human gene encoding the human type VI adenylyl cyclase, methods for the recombinant production of purified human type VI adenylyl cyclase and the proteins made by these methods, antibodies against the whole human type VI adenylyl cyclase or regions thereof, vectors, nucleotide probes, and host cells transformed by genes encoding polypeptides having human type VI adenylyl cyclase activity, along with diagnostic and therapeutic uses for these various reagents.

Background of the Invention

Adenylyl cyclases direct the intracellular synthesis of the primary second messenger, cyclic-3',5'-adenosine monophosphate (cAMP), by converting ATP to cAMP, principally in response to a diverse family of membrane spanning, G-protein coupled receptors, each activated by its own extracellular hormone or protease. Signal transduction for G-protein coupled receptors occurs through a coupled heterotrimeric G protein complex composed of the alpha (G_α), and beta/gamma ($G_{\beta\gamma}$) subunits. Upon receptor stimulation, G_α exchanges GTP for GDP, dissociates from both $G_{\beta\gamma}$ and the receptor, and proceeds to directly regulate various effectors, including adenylyl cyclase. Multiple families of G_α proteins have been identified, two of which are named for their effects on regulating adenylyl cyclase activity ($G_{\alpha s}$ family stimulates all adenylyl cyclases, while $G_{\alpha i}$ family inhibits most but not all of the adenylyl cyclases). Each of these G_α proteins has its own tissue distribution, and subset of coupled receptors, which favors receptor specific regulation of adenylyl cyclase.

Additional studies have suggested other means by which adenylyl cyclase activity may be regulated within tissues. This concept is derived from findings that a number of adenylyl cyclase isoforms exist, each with their own gene locus, distinct set of responses to intracellular signals and unique tissue distribution. To date, nine separate isoforms (Types I-IX) have been characterized, principally from rodents, each with its own regulatory properties and tissue specific distribution.

The structure of adenylyl cyclases has been greatly studied and the putative domains given standard nomenclature. Topographically, the adenylyl cyclase isoforms are similar, having two six-transmembrane spanning regions associated with an intracellular N-terminus, a large cytoplasmic loop (ICD III, more commonly referred to as "C_1") and an intracellular C-terminus (more commonly referred to as "C_2"). The transmembrane region between the N-terminus and the C_1 loop is commonly referred to as "M1". The M1 region has three extracellular domains (ECD I, II and III), two intracellular domains (ICD I and II) and six transmembrane domains (TM I, II, III, IV, V and VI). The region between the C_1 loop and the C-terminus is referred to as "M2". The M2 region has three extracellular domains (ECD IV, V and VI), two intracellular domains (ICD IV and V) and six transmembrane domains (TM VII, VIII, IX, X, XI and XII). The N-terminus is commonly divided into two regions, designated "N_1" and "N_2". The large C_1 cytoplasmic loop is also divided into two regions, a long "C_{1a}" region and a shorter "C_{1b}" region. Lastly, the C-terminus is divided into a long "C_{2a}" region and a shorter "C_{2b}" region. An extensive discussion of these regions can be found in Broach, *et al.*, WO 95/30012, which is incorporated herein by reference. The amino acid sequence of the C_{1a} and C_{2a} regions are conserved among the different isoforms. On the other hand, the N-terminus, C_{1b} and C_{2b} regions show the most diversity among the various isoforms.

Based on sequence and functional similarities, these isoforms fall into six distinct classes of adenylyl cyclases. Type VI is in the same class as type V, showing sequence similarity even in the transmembrane regions where the greatest level of divergence is noted among the isoforms. Type V is predominantly expressed in heart and brain tissue. Type VI has a somewhat broader distribution, but its dominant expression is also in heart and brain tissue. Type VI, like type V, has a relatively longer N-terminus and relatively

shorter C-terminus, lacking the C_{2b} region, than the other isoforms.

Diversity in activities, and differences in distribution and prevalence of adenylyl cyclase isoforms, may contribute to tissue specific regulation of cAMP levels. It is expected that by taking advantage of distinct structural and biochemical differences between different adenylyl cyclases, isoform specific or selective modulators can be discovered. This, in conjunction with knowledge of the proportion and distribution of each isoform in select tissues provides a means by which one can develop either tissue specific, or selective pharmacological agents since it is expected that isoform specific modulators would have tissue specificity related to the distribution of that isoform.

Key to the development of selective pharmacological agents is information pertaining to the tissue specific distribution and prevalence of each isoform. To date most of this information is available for isoform mRNA levels in a handful of non-human mammals, although some select mRNA (e.g. Type V) have been measured for many human tissues. Acquiring information on protein isoform distribution in human tissues is considered an important aspect of pharmaceutical research in this area, since this could either strengthen existing target information or point to different isoforms, when compared with mRNA data.

To date, only three full length human adenylyl cyclase isoforms have been cloned: Type II adenylyl cyclase (Stengel, *et al.*, Hum. Genet. 90:126-130 (1992)), Type VII adenylyl cyclase (Nomura, *et al.*, DNA Research 1:27-35 (1994)) and Type VIII adenylyl cyclase (Defer, *et al.*, FEBS Letters 351:109-113 (1994)).

Type VI has been cloned from mouse NCB-20 cells (Yoshimura, *et al.*, Proc. Natl. Acad. Sci. USA 89:6716-6720 (1992)) and canine heart (Katsushika, *et al.*, Proc. Natl. Acad. Sci. USA 89:8774-8778 (1992) and Ishikawa, U.S. Patent No. 5,334,521). The human isoform has not been cloned until now.

Summary Of The Invention

One aspect of the invention is an isolated and purified human type VI adenylyl cyclase (hAC6) polypeptide comprising the amino acid sequence of Figure 1 (SEQ ID NO:2).

Another aspect of the invention is an isolated and purified nucleic acid encoding for the hAC6 polypeptide.

Yet another aspect of the invention is an isolated and purified nucleic acid comprising the nucleotide sequence of Figure 1 (SEQ ID NO:1) , which encodes a biologically active hAC6 polypeptide, or fragment thereof.

Still another aspect of the invention is an isolated and purified nucleic acid comprising the nucleotide sequence of Figure 1 (SEQ ID NO:1) , which encodes a biologically active soluble hAC6 peptide fragment.

Another aspect of the present invention also relates to the human gene encoding human type VI adenylyl cyclase, which has both diagnostic and therapeutic uses as are described below. Included within this invention are proteins or peptides having substantial homology with proteins or peptides comprising the amino acid sequence of Figure 1 or encoded by a gene having substantial homology with the nucleotide sequence of Figure 1, and which exhibit the same characteristics of human type VI adenylyl cyclase.

Yet another aspect of the invention is a method of producing hAC6 which comprises incorporating a nucleic acid having the nucleotide sequence of Figure 1 (SEQ ID NO:1) into an expression vector, transforming a host cell with the vector and culturing the transformed host cell under conditions which result in expression of the gene.

Brief Description Of The Drawings

Figure 1(A-I) is the DNA (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of human type VI adenylyl cyclase. The entire coding sequence, as well as portions of the 5' and 3' untranslated sequences, are shown. The whole sequence was done bidirectionally twice by dideoxy sequencing method using Taq polymerase.

Detailed Description Of The Invention

Definitions

Before proceeding further with a description of the specific embodiments of the present invention, a number of terms will be defined:

The terms "substantially pure" and "isolated" are used herein to describe a protein that has been separated from the native contaminants or components that naturally accompany it. Typically, a monomeric protein is substantially pure when at least about 60 to 70% of a sample exhibits a single polypeptide backbone. Minor variants or chemical modifications typically share approximately the same polypeptide sequence. A substantially pure protein will typically comprise over about 85 to 90% of a protein sample, preferably will comprise at least about 95%, and more preferably will be over about 99% pure. Purity is typically measured on a polyacrylamide gel, with homogeneity determined by staining. For certain purposes, high resolution will be desired and HPLC or a similar means for purification utilized. However, for most purposes, a simple chromatography column or polyacrylamide gel will be used to determine purity. Whether soluble or membrane bound, the present invention provides for substantially pure preparations. Various methods for their isolation from biological material may be devised, based in part upon the structural and functional descriptions contained herein. In addition, a protein that is chemically synthesized or synthesized in a cellular system that is different from the cell from which it naturally originates, will be substantially pure. The term is also used to describe proteins and nucleic acids that have been synthesized in heterologous mammalian cells, bacterial cells such as *E. coli* and other prokaryotes.

As used herein, the terms "hybridization" (hybridizing) and "specificity" (specific for) in the context of nucleotide sequences are used interchangeably. The ability of two nucleotide sequences to hybridize to each other is based upon a degree of complementarity of the two nucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence that are complementary to another sequence, the greater the degree of hybridization of one to the other. The degree of hybridization also depends on the conditions of stringency which include temperature, solvent ratios, salt concentrations, and the like. In particular, "selective hybridization" pertains to conditions in which the degree of hybridization of a polynucleotide of the invention to its target would require complete or nearly complete complementarity. The complementarity must be sufficiently high so as to assure that the polynucleotide of the invention will bind specifically to the target relative to binding other nucleic acids present in the hybridization medium. With selective

hybridization, complementarity will be 90-100%, preferably 95-100%, more preferably 100%.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium titrate/0.1% $NaDodSO_4$ at 50°C, or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin ("BSA")/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x 0.75 M NaCl and 0.075 M sodium citrate ("SSC"), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 mg/ml), 0.1% sodium dodecyl sulfate ("SDS"), and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

"Isolated" nucleic acid will be nucleic acid that is identified and separated from contaminant nucleic acid encoding other polypeptides from the source of nucleic acid. The nucleic acid may be labeled for diagnostic and probe purposes, using any label known and described in the art as useful in connection with diagnostic assays.

Preferred Embodiments

The present invention relates to human type VI adenylyl cyclase, which is referred to herein as "hAC6". Figure 1 shows the DNA sequence of the clone encoding the hAC6 polypeptide along with the deduced amino acid sequence. As used herein, the terms "hAC6 polypeptide" or "hAC6 enzyme" refer to any adenylyl cyclase sharing a common biological activity with the human type VI adenylyl cyclase contained in the clone described in Example 1. This "common biological activity" includes but is not limited to an effector function or cross-reactive antigenicity.

As indicated above, type VI adenylyl cyclase is in the same isoform class as type VI, being expressed mainly in the heart and brain. As with the other known isoforms, type VI adenylyl cyclase has a similar putative structure: six extracellular domains; five intracellular domains, four small ones and a large cytoplasmic loop; and intracellular amino and carboxy termini.

However, type VI adenylyl cyclase, like type V, is distinguishable over other adenylyl cyclase isoforms in that it has a larger N-terminus and a relatively shorter C-terminus as it lacks the C_{2b} region. In the other mammalian isoforms (types I-V and VII-IX), much of the membrane associated secondary structure is well conserved. Certain portions of the hAC6 polypeptide are similarly conserved.

The scope of the present invention is not limited to the exact sequence of the hAC6 cDNA set forth in Figure 1 (SEQ ID NO: 1), or the use thereof. The invention contemplates certain modifications to the sequence, including deletions, insertions, and substitutions, such as are well known to those skilled in the art. For example, the invention contemplates replacing one or more codons in the cDNA sequence of Figure 1, with codons that encode amino acids that are chemically equivalent to the amino acids in the native protein. Chemical equivalency is determined, for example, by one or more of the following characteristics: hydrophobicity or hydrophilicity, charge, size, whether the residue is cyclic or non-cyclic, aromatic or non-aromatic. So, for example, a codon encoding a neutral polar amino acid can be substituted with another codon that encodes a neutral polar residue, with the reasonable expectation of producing a biologically equivalent product.

Amino acid residues can be generally classified into four groups. Acidic residues are hydrophilic and have a negative charge due to loss of H^+ at physiological pH. Basic residues are also hydrophilic but have a positive charge due to association with H^+ at physiological pH. Neutral nonpolar residues are hydrophobic and are not charged at physiological pH. Neutral polar residues are hydrophilic and are not charged at physiological pH. Amino acid residues can be further classified as cyclic or noncyclic, and aromatic or nonaromatic, self-explanatory classifications with respect to the side chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of 4 carbon atoms or less, inclusive of the carboxyl carbon. Small residues are, of course, always nonaromatic.

Of the naturally occurring amino acids, aspartic acid and glutamic acid are acidic; arginine and lysine are basic and noncyclic; histidine is basic and cyclic; glycine, serine and cysteine are neutral, polar and small; alanine is neutral, nonpolar and small;

threonine, asparagine and glutamine are neutral, polar, large and nonaromatic; tyrosine is neutral, polar, large and aromatic; valine, isoleucine, leucine and methionine are neutral, nonpolar, large and nonaromatic; and phenylalanine and tryptophan are neutral, nonpolar, large and aromatic. Proline, although technically neutral, nonpolar, large, cyclic and nonaromatic, is a special case due to its known effects on the secondary conformation of peptide chains, and is not, therefore, included in this defined group.

There are also commonly encountered amino acids, which are not encoded by the genetic code. These include, by way of example and not limitation: sarcosine, beta-alanine, 2,3-diamino propionic and alpha-aminisobutyric acid which are neutral, nonpolar and small; *t*-butylalanine, *t*-butylglycine, N-methylisoleucine, norleucine and cyclohexylalanine which are neutral, nonpolar, large and nonaromatic; ornithine which is basic and noncyclic; cysteic acid which is acidic; citrulline, acetyl lysine, and methionine sulfoxide which are neutral, polar, large and nonaromatic; and phenylglycine, 2-naphthylalanine, β-2-thienylalanine and 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid which are neutral, nonpolar, large and aromatic.

Ordinarily, the hAC6 polypeptide claimed herein will have an overall amino acid sequence having at least 75% amino acid sequence identity with the hAC6 sequence disclosed in Figure 1, more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. More particularly, the N-terminus, C1b and C2b regions of the hAC6 polypeptide or polypeptide fragment claimed herein, will have an amino acid sequence having at least 90%, and most preferably at least 95% amino acid sequence identity with the hAC6 sequence disclosed in Figure 1. Identity or homology with a sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the sequence of the hAC6 polypeptide, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. N-terminal, C-terminal or internal extensions, deletions, or insertions of the hAC6 sequence shall be construed as affecting homology.

Thus, the claimed hAC6 polypeptide that is the subject of this invention includes molecules having the hAC6 amino acid sequence; fragments thereof having a consecutive sequence of at least 10, 15, 20, 25, 30 or 40 amino acid residues from the hAC6

sequence of Figure 1, which exhibits the hAC6 polypeptide characteristics; amino acid sequence variants of the hAC6 sequence of Figure 1 wherein an amino acid residue has been inserted N- or C-terminal to, or within, (including parallel deletions) the hAC6 sequence or its fragments as defined above; amino acid sequence variants of the hAC6 sequence of Figure 1 or its fragments as defined above which have been substituted by at least one residue, and which exhibit the hAC6 polypeptide characteristics. Of particular interest are those peptides corresponding to those regions where the hAC6 polypeptide is divergent from types I-V and VII-IX.

Human type VI adenylyl cyclase polypeptides include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis; naturally occurring variants of the hAC6 polypeptide; derivatives of the hAC6 polypeptide or its fragments wherein the hAC6 or its fragments have been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope); glycosylation variants of the hAC6 (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of appropriate amino acid); and soluble forms of the hAC6 polypeptide or fragments thereof. This invention also includes tagging the hAC6 polypeptide, for example, for use in a diagnostic application. Types and methods of tagging are well known in the art, for example, the use of hexa-histidine tags.

Several regions of the Type VI isoform are highly conserved with the other adenylyl cyclase isoforms. Accordingly, it is believed that most sequence modifications to the highly conserved regions such as the extracellular domains, transmembrane regions and short intracellular domains, including deletions and insertions, and substitutions in particular, are not expected to produce radical changes in the characteristics of the hAC6 polypeptide, distinct from those found with similar changes to other isoforms. However, when it is difficult to predict the exact effect of the sequence modification in advance of making the change, one skilled in the art will appreciate that the effect of any sequence modification will be evaluated by routine screening assays.

The nomenclature used to describe the peptide compounds of the invention follows the conventional practice where the N-terminal amino group is assumed to be to the left

and the carboxy group to the right of each amino acid residue in the peptide. In the formulas representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although often not specifically shown, will be understood to be in the form they would assume at physiological pH values, unless otherwise specified. Thus, the N-terminal H^+_2 and C-terminal O^- at physiological pH are understood to be present though not necessarily specified and shown, either in specific examples or in generic formulas. Free functional groups on the side chains of the amino acid residues can also be modified by amidation, acylation or other substitution, which can, for example, change the solubility of the compounds without affecting their activity. All of the compounds of the invention, when an amino acid forms the C-terminus, may be in the form of the pharmaceutically acceptable salts or esters. Salts may be, for example, Na^+, K^+, Ca^{+2}, Mg^{+2} and the like; the esters are generally those of alcohols of 1-6 carbons.

In all of the peptides of the invention, one or more amide linkages (-CO-NH-) may optionally be replaced with another linkage which is an isostere such as $-CH_2NH-$, $-CH_2S-$, $-CH_2CH_2$, -CH=CH- (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2SO-$. This replacement can be made by methods known in the art. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, Vega Data 1(3) "Peptide Backbone Modifications" (general review) (March 1983); Spatola, in "Chemistry and Biochemistry of Amino Acids Peptides and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983) (general review); Morley, J.S., Trends Pharm Sci , pp. 463-468 (general review) (1980); Hudson, *et al.*, Int J Pept Prot Res 14:177-185 ($-CH_2NH-$, $-CH_2CH_2-$) (1979) ; Spatola, *et al.*, Life Sci 38:1243-1249 ($-CH_2-S$) (1986); Hann, J Chem Soc Perkin Trans I 307-314 (-CH-CH-, cis and trans) (1982); Almquist, *et al.*, J Med Chem 23:1392-1398 ($-COCH_2-$) (1980); Jennings-White, *et al.*, Tetrahedron Lett 23:2533 ($-COCH_2-$) (1982); Szelke, *et al.*, European Application EP 45665 (1982) CA:97:39405 (1982) ($-CH(OH)CH_2-$); Holladay, *et al.*, Tetrahedron Lett 4:4401-4404 ($-C(OH)CH_2-$) (1983); and Hruby, Life Sci 31:189-199 ($-CH_2-S-$) (1982).

Human type VI adenylyl cyclase peptides may be purified using techniques of classical protein chemistry, such as are well known in the art. For example, a lectin affinity chromatography step may be used, followed by a highly specific ligand affinity

chromatography procedure that utilizes a ligand conjugated to biotin through the cysteine residues of the ligand. Alternately, the hexa-histidine tagged hAC6 polypeptide may be purified using nickel column chromatography.

One embodiment of the invention relates to recombinant materials associated with the production of the hAC6 polypeptide. One method of producing hAC6 comprises incorporating a nucleic acid having the nucleotide sequence of Figure 1 (SEQ ID NO:1) into an expression vector, transforming a host cell with the vector and culturing the transformed host cell under conditions which result in expression of the gene. Suitable expression vectors include pc3hAC6. Examples of host cells includes bacterial, viral, yeast, insect or mammalian cell lines. A preferred host cell is the human embryonic cell line referred to as "HEK-293".

The invention also contemplates the use of transfected cells that can be cultured so as to display or express hAC6 on its surface, thus providing an assay system for the interaction of materials with the native hAC6 where these cells or relevant fragments of hAC6 are used as a screening tool to evaluate the effect of various candidate compounds on hAC6 activity *in vivo*, as is described below. Another embodiment of the invention relates to recombinant materials associated with the production of soluble hAC6 fragments. These include transfected cells, such as *E. coli*, that can be cultured so as to express active portions of the hAC6 polypeptide, in particular the C1 and C2 (C-terminus) intracellular loops. These soluble fragments can be purified and reconstituted to obtain enzymatic activity. This has been demonstrated with like domains from other isoforms. See Whisnant, *et al.*, Proc. Natl. Acad. Sci.:93:6621-6625 (1996). Such soluble fragments can also be used as a screening tool to evaluate the effect of various candidate compounds on hAC6 activity. Suitable cells for transfection include bacterial cells, insect cells such as Sf-9 cells, yeast cells and most mammalian cell lines.

Recombinant production of the hAC6 polypeptide involves using a nucleic acid sequence that encodes hAC6, as is set forth in Figure 1, or its degenerate analogs. The nucleic acid can be prepared either by retrieving the native sequence, as described below, or by using substantial portions of the known native sequence as a probe, or it can be synthesized *de novo* using procedures that are well known in the art.

The nucleic acid may be ligated into expression vectors suitable for the desired host and then transformed into compatible cells. Suitable vectors suitable for use in transforming bacterial cells are well known in the art. Plasmids and bacteriophages, such as lambda phage, are commonly used as vectors for bacterial hosts such as *E. coli*. Virus vectors are suitable for use in mammalian and insect cells for expression of exogenous DNA. Mammalian cells are readily transformed with SV40 or polyoma virus; and insect cells in culture may be transformed with baculovirus expression vectors. Suitable yeast vector systems include yeast centromere plasmids, yeast episomal plasmids and yeast integrating plasmids. Alternatively, nucleic acids may be introduced directly into a host cell by techniques such as are well known in the art.

The cells are cultured under conditions favorable for the expression of the gene encoding the hAC6 polypeptide and cells displaying hAC6 on the surface are then harvested. Suitable eukaryotic host cells include mammalian cells, plant cells, yeast cells and insect cells. Suitable prokaryotic host cells, include bacterial cells such as *E. coli* and *Bacillus subtilis*, Chinese Hamster Ovary cells, COS cells, the rat-2 fibroblast cell line, the human embryonic kidney 293 cell line, and insect cell lines such as Sf-9.

This invention also relates to nucleic acids that encode or are complementary to a hAC6 polypeptide. These nucleic acids can then be used to produce the polypeptide in recombinant cell culture for diagnostic use or for potential therapeutic use. In still other aspects, the invention provides an isolated nucleic acid molecule encoding hAC6, either labeled or unlabeled, or a nucleic acid sequence that is complementary to, or hybridizes under stringent conditions to, a nucleic acid sequence encoding hAC6. The isolated nucleic acid molecule of the invention excludes nucleic acid sequences which encode, or are complementary to nucleic acid sequences encoding, other known adenylyl cyclase isoforms.

This invention also provides a replicable vector comprising a nucleic acid molecule encoding hAC6 operably linked to control sequences recognized by a host transformed by the vector; host cells transformed with the vector; and a method of using a nucleic acid molecule encoding hAC6 to effect the production of hAC6 on the cell surface or as soluble fragments, comprising expressing the nucleic acid molecule in a culture of the

transformed host cells and recovered from the cells. The nucleic acid sequence is also useful in hybridization assays for hAC6-encoding nucleic acid molecules.

In still further embodiments of the invention, a method is described for producing hAC6 comprising inserting into the DNA of a cell containing the nucleic acid sequence encoding hAC6, a transcription modulatory element (such as an enhancer or a silencer) in sufficient proximity and orientation to the hAC6-coding sequence to influence transcription thereof, with an optional further step comprising culturing the cell containing the transcription modulatory element and the hAC6-encoding nucleic acid sequence.

This invention also covers a cell comprising a nucleic acid sequence encoding the hAC6 polypeptide and an exogenous transcription modulatory element in sufficient proximity and orientation to the above coding sequence to influence transcription thereof and a host cell containing the nucleic acid sequence encoding hAC6 operably linked to exogenous control sequences recognized by the host cell.

This invention provides a method for obtaining cells having increased or decreased transcription of the nucleic acid molecule encoding the hAC6 polypeptide, comprising: providing cells containing the nucleic acid molecule; introducing into the cells a transcription modulating element; and screening the cells for a cell in which the transcription of the nucleic acid molecule is increased or decreased.

Human adenylyl cyclase type VI nucleic acids for use in the invention can be produced as follows. A hAC6 "nucleic acid" is defined as RNA or DNA that encodes the hAC6 polypeptide, or is complementary to nucleic acid sequence encoding hAC6, or hybridizes to such nucleic acid and remains stably bound to it under stringent conditions, or encodes a polypeptide sharing at least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the deduced amino acid sequence shown in Figure 1. It is typically at least about 10 nucleotides in length and preferably has hAC6 related biological or immunological activity. Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbone or including alternative bases whether derived from natural sources or synthesized.

Of particular interest is a hAC6 nucleic acid that encodes a full-length molecule, including but not necessarily the native signal sequence thereof. Nucleic acid encoding full-length protein is obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures to secure DNA that is complete at its 5' coding end. Such a clone is readily identified by the presence of a start codon in reading frame with the original sequence.

DNA encoding an amino acid sequence variant of the hAC6 polypeptide is prepared as described below or by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of hAC6.

Techniques for isolating and manipulating nucleic acids are disclosed for example by the following documents: U.S. Patent No. 5,030,576, U.S. Patent No. 5,030,576 and International Patent Publications WO94/11504 and WO93/03162. See, also, Sambrook, *et al.*, "Molecular Cloning: A Laboratory Manual", 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989, and Ausubel, *et al.*, "Current Protocols in Molecular Biology", Vol. 2, Wiley-Interscience, New York, 1987.

The isolation, recombinant production and characterization of the hAC6 polypeptide allows for the design of assay systems using hAC6. The availability of the isolated cells providing hAC6 on their surface and the availability of the recombinant DNA encoding hAC6 which permits display and expression of the enzyme on host cell surfaces, all makes such cells available as a valuable tool for evaluating the ability of candidate pharmaceuticals, both agonists and antagonists, to affect the activity of hAC6. In this manner, the invention is related to assay systems which utilize isolated or recombinantly produced hAC6 to screen for agonist and antagonist activity of candidate drugs. This assay is especially useful in assuring that these candidate therapeutic agents have the desired effect on hAC6. Determination of these properties is essential in evaluating the specificity of drugs for other adenylyl cyclase isoforms.

The host cells are typically animal cells, most typically mammalian cells. In order to be useful in the assays, the cells must have intracellular mechanisms which permit hAC6 to be displayed on the cell surface or to be expressed as soluble fragments. The animal host cells expressing the DNA encoding the hAC6 polypeptide or a fragment thereof are then cultured to effect the expression of the encoding nucleic acids so as to either 1) produce hAC6 display on the cell surface such that the cells can then be used directly in assays for assessment of a candidate drug to bind to or otherwise affect the activity of the enzyme, or 2) produce hAC6 as soluble fragments which can then be purified and reconstituted to obtain an enzymatically active compound useful in screening assays.

There are several possible strategies to identify compounds which affect hAC6 activity. Over expression of the hAC6 cDNA can provide a means for isolation of large quantities of crude membrane preparations from a stable cell line. HEK-293 cells have been found to be particularly useful for this purpose. In this system the measurable enzyme activity would be predominantly from expression of recombinant hAC6. A highly sensitive, reproducible, high throughput screening system is desirable, with enzyme activity detected in a 96 well, scintillation proximity-type assay to measure product formation (cAMP). There are numerous screening assays that can be utilized. For example, the basal (unstimulated) activity of hAC6 can be measured as a method of detecting both agonists and antagonists of the hAC6 enzyme. In addition, stimulation of the enzyme by its most relevant physiological activator, the heterotrimeric G protein subunit, $G_{\alpha s}$, can be assayed using activated (GTPγS bound) recombinant bovine $G_{\alpha s}$ (expressed and purified from bacteria), with the expectation that additional compounds may be identified which inhibit $G_{\alpha s}$ stimulation of the hAC6 polypeptide. Other stimulatory agents can also be used, such as forskolin or forskolin analogs. "Hits", i.e., compounds which affect hAC6, in any of these screens will be further evaluated in other assays to help focus on compounds which are relevant to the targeted isoform.

Another method of evaluating candidates as potential therapeutic agents typically involves a screening based approach such as a binding assay in which the candidate (such as a peptide or a small organic molecule) would be tested to measure if, or to what extent, it binds the catalytic subunit of the hAC6 enzyme. Preferably, a mammalian cell line that

expresses recombinant hAC6 or plasma membrane preparations thereof, will be used in the assay. For example, a candidate antagonist competes for binding to hAC6 with either a labeled agonist or antagonist, for example labeled forskolin or a labeled forskolin analog. Varying concentrations of the candidate are supplied, along with a constant concentration of the labeled agonist or antagonist. The inhibition of binding of the labeled material can then be measured using established techniques. This measurement is then correlated to determine the amount and potency of the candidate that is bound to hAC6.

Another method of identifying compounds which affect hAC6 activity is the rational design of synthetic compounds based on nucleotide scaffolds, targeted to either of two distinct sites on the hAC6 enzyme. One of these is the active site (ATP being the substrate, cAMP being the product) and the other is the separate P site (adenine nucleoside 3'-polyphosphates reportedly demonstrating the greatest inhibitory activity, with either pure or crude enzyme preparations). As a related approach, one could attempt to design forskolin analogues which may demonstrate isoform specific effects.

In addition, using the above assays, the ability of a candidate drug to stimulate or inhibit the activity of hAC6 can be tested directly.

Once lead candidates are identified, and for purposes of demonstrating that isoform specificity may be achieved with small molecule modulators, it is desirable to develop assay systems which monitor most, and preferably all, human adenylyl cyclase isoforms. These assays may be used to evaluate either existing (e.g. forskolin analogs or P site inhibitors) or newly discovered small molecule modulators and determine structure activity relationships for different adenylyl cyclase isoforms. Such assays could also be used to evaluate either specific or selective modulators of other adenylyl targets and with use of a whole cell assay, may provide useful insights for designing bioavailability and addressing biological activity of lead candidates.

The hAC6 also has utility in assays for the diagnosis of diseases and disorders by detection, in tissue samples, of aberrant expression of the hAC6 enzyme.

Another aspect of the invention relates to hAC6 agonists that imitate the naturally occurring form of hAC6. These agonists are useful as control reagents in the

above-mentioned assays to verify the workability of the assay system. In addition, agonists for hAC6 may exhibit useful effects *in vivo* in treating disease.

Another aspect of the invention relates to hAC6 antagonists that are modified forms of hAC6 peptides. Such antagonists bind to hAC6, and prevent enzyme-substrate interaction by blocking their binding to hAC6. Another group of compounds within the scope of the invention, are antagonists of hAC6 substrate, i.e., these are substrate inhibitors. Both these types of antagonists find utility in diminishing or mediating events based upon enzyme-substrate interaction such as cAMP production. Yet another second group of antagonists includes antibodies designed to bind specific portions of hAC6. In general, these are monoclonal antibody preparations which are highly specific for any desired region of hAC6, although polyclonal antibodies are also contemplated by this invention. The antibodies, which are explained in greater detail below, are also useful in immunoassays for the hAC6 enzyme, for example, in assessing successful expression of the gene in recombinant systems.

In both the agonists and antagonists, a preferred embodiment is that class of compounds having amino acid sequences that are encoded by the hAC6 gene. The invention also includes those compounds where one, two, three or more of said amino acid residues are replaced by one(s) which is not encoded genetically. Also included in the invention are isolated DNA molecules that encode these specific peptides.

It is believed that the extracellular domains of enzymes may play a key role in extracellular activities, for example, in enzyme regulation. Accordingly, the invention includes agonists and antagonists having amino acid sequences, in whole or in part, corresponding to the extracellular domains of hAC6, the sequences of which can be approximated from the amino acid sequence of Figure 1 and the hydropathy analysis of Figure 4. The invention also includes agonists and antagonists that affect the enzyme's function by binding to the N- or C-terminus or to one of the intracellular (ICD) domains of hAC6, the sequences of which can be approximated from the amino acid sequence of Figure 1 and the hydropathy analysis.

In other adenylyl cyclases, the ICD IV and carboxy terminus regions have been shown to play a role in enzyme activity or G_α or forskolin interaction. See for example: Whisnant, *et al.*, *supra*. Accordingly, it is expected that the amino acid sequences of the

ICD IV and carboxy terminus regions of hAC6, in whole or in part, will be particularly useful in designing antibodies or peptides that can bind the enzyme and block enzyme activity or $G_{\alpha s}$ interaction.

As the understanding of adenylyl cyclases and factors which effect isoform activity increases, rational drug design is becoming a viable alternative in pharmaceutical research. It is believed that the two conserved intracellular domains of adenylyl cyclase (the C_1 and C_2 domains) associate to form an active enzyme. This has been demonstrated with studies that combine both expressed recombinant C_1 and C_2 domains. Both the C_1 and C_2 domains are required to reconstitute enzyme activity while either alone has no substantial activity. Forskolin plus $G_{\alpha s}$ stimulates this system, by increasing the association of the two domains. Designing assays which monitor enzyme activity, dependent on association of two separate domains, is expected to provide greater sensitivity to antagonists since this would presumably be more easily disrupted. Other studies have demonstrated that peptides, comprised of sequences from conserved regions of the intracellular domains, act as inhibitors of detergent solubilized enzyme preparations. This invention contemplates the use of peptide walking strategies, to delimit regions of the modulator which may be responsible for its activity, leading to the design of small molecule inhibitors. Finally, knowledge of uncharacterized, physiological modulators of adenylyl cyclase, particularly those that demonstrate isoform specificity, may provide new assay systems for identifying novel AC modulators. It is expected that many of these modulators would be proteins and some may be identified while using adenylyl cyclase sequences as "bait" in a yeast two hybrid system. Alternatively one may identify proteins which coprecipitate with adenylyl cyclase upon capture with adenylyl cyclase antibodies.

The peptide agonists and antagonists of the invention are preferably about 10-100 amino acids in length, more preferably 25-75 amino acids in length. These peptides can be readily prepared using standard solid phase or solution phase peptide synthesis, as is well known in the art. In addition, the DNA encoding these peptides can be synthesized using commercially available oligonucleotide synthesis instrumentation and recombinantly produced using standard recombinant production systems. Production using solid phase

peptide synthesis is required when non-gene encoded amino acids are to be included in the peptide.

Another aspect of the invention pertains to antibodies, which have both diagnostic and therapeutic uses. Antibodies are able to act as antagonists or agonists by binding specific regions of the hAC6 polypeptide. These antibodies also find utility in immunoassays that measure the presence of hAC6, for example in immunoassays that measure gene expression. In general, antibodies to adenylyl cyclases, and more importantly, those which may recognize specific isoforms of adenylyl cyclase, are a useful tool to evaluate tissue distribution and prevalence of the adenylyl cyclase protein. By identifying regions of dissimilarity between the adenylyl cyclase isoforms and the antigenic potential of these regions, either synthetic peptides or recombinant proteins to these sequences can be created for use in immunization. The resulting antibodies would then be characterized for specificity based on the unique qualities of the immunogen and reactivity with other expressed isoforms. Detection of isoform protein in various tissues can readily be monitored by Westerns blots; however, immunohistochemical analysis would also be useful. This information is useful to identify the adenylyl cyclase target of interest, providing valuable insights into useful therapeutic strategies such as targets in cardiovascular disease, asthma or obesity.

The antibodies of the present invention can be prepared by techniques that are well known in the art. The antibodies can be monoclonal or polyclonal, but are preferably monoclonal antibodies that are highly specific for hAC6 and can be raised against the whole hAC6 polypeptide or regions thereof. Antibodies are prepared by immunizing suitable mammalian hosts (typically rabbit, rat, mouse, goat, human, etc.) in appropriate immunization protocols using the peptide haptens (immunogen) alone, if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. The immunogen will typically contain a portion of the hAC6 polypeptide that is intended to be targeted by the antibodies. Critical regions include those regions corresponding to the extracellular domains of the hAC6 enzyme, any region(s) of proteolytic cleavage, and any segment(s) of the extracellular segment critical for activation. Methods for preparing immunogenic conjugates with carriers such as bovine serum albumin, keyhole limpet hemocyanin, or other carrier proteins are well

known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the hapten. The hapten can be extended at the amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to carrier. The desired immunogen is administered to a host by injection over a suitable period of time using suitable adjuvants followed by collection of sera. Over the course of the immunization schedule, titers of antibodies are taken to determine the adequacy of antibody formation.

Polyclonal antibodies are suitable for many diagnostic and research purposes and are easily prepared. Monoclonal antibodies are often preferred for therapeutic applications and are prepared by continuous hybrid cell lines and collection of the secreted protein. Immortalized cell lines that secrete the desired monoclonal antibodies can be prepared by the method described in Kohler and Milstein, Nature 256:495-497 (1975) or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines are then screened by immunoassay techniques in which the antigen is the immunogen or a cell expressing hAC6 on its surface. Cells that are found to secrete the desired antibody, can then be cultured *in vitro* or by production in the ascites fluid. The antibodies are then recovered from the culture supernatant or from the ascites supernatant.

Alternately, antibodies can be prepared by recombinant means, i.e., the cloning and expression of nucleotide sequences or mutagenized versions thereof that at a minimum code for the amino acid sequences required for specific binding of natural antibodies. Antibody regions that bind specifically to the desired regions of hAC6 can also be produced as chimeras with regions of multiple species origin.

Antibodies may include a complete immunoglobulin or a fragment thereof, and includes the various classes and isotypes such as IgA, IgD, IgE, IgG1, IgG2a, IgG2b, IgG3 and IgM. Fragments include Fab, Fv, $F(ab')_2$, Fab', and so forth. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', or $F(ab')_2$ fragments is often

preferable, especially in a therapeutic context, as these fragments have different immunogenicity than the whole immunoglobulin, and do not carry the biological activity of an immunoglobulin constant domain.

The antibodies thus produced are useful not only as potential agonist or antagonists for the hAC6 polypeptide, filling the role of agonist or antagonist in the assays of the invention, but are also useful in immunoassays for detecting the hAC6 enzyme. As such these antibodies can be coupled to imaging agents for administration to a subject to allow detection of localized antibody to ascertain the under-or over-expression of hAC6 in tissues of interest. In addition, these reagents are useful in vitro to detect, for example, the successful production of hAC6 on the surface of the recombinant host cells.

Yet another aspect of the invention relates to pharmaceutical compositions containing the compounds and antibodies of the invention. The agonists and antagonists of the invention have therapeutic utility in (1) treating diseases caused by aberrant activity of the hAC6 enzyme in tissues where it is customarily found, for example in the heart and brain and (2) treating diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of hAC6.

The peptide agonists and antagonists of the invention can be administered in conventional formulations for systemic administration such as is well known in the art. Typical formulations may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition.

Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can also be used. More recently, alternative means for systemic administration of peptides have been devised which include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the patient's condition, and the judgment of the attending physician. Suitable dosage ranges, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of peptides available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art.

The invention also relates to the therapeutic, prophylactic and research uses of various techniques to block or modulate the expression of the hAC6 by interfering with the transcription of translation of a DNA or RNA molecule encoding the hAC6. This includes a method to inhibit or regulate expression of hAC6 in a cell comprising providing to the cell an oligonucleotide molecule which is antisense to, or forms a triple helix with, hAC6-encoding DNA or with DNA regulating expression of hAC6-encoding DNA, in an amount sufficient to inhibit or regulate expression of the hAC6, thereby inhibiting or regulating its expression. Also included is a method to inhibit or regulate expression of hAC6 in a subject, comprising administering to the subject an oligonucleotide molecule which is antisense to, or forms a triple helix with, hAC6-encoding DNA or with DNA regulating expression of hAC6-encoding DNA, in an amount sufficient to inhibit or regulate expression of hAC6 in the subject, thereby inhibiting or regulating its expression. The antisense molecule or triple helix-forming molecule in the above methods is preferably a DNA or RNA oligonucleotide. These utilities are described in greater detail below.

The constitutive expression of antisense RNA in cells has been shown to inhibit the expression of about 20 different genes in mammals and plants, and the list continually grows (Hambor, *et al.*, J. Exp. Med. 168:1237-1245 (1988); Holt, *et al.*, Proc. Natl. Acad. Sci. 83:4794-4798 (1986); Izant, *et al.*, Cell 36:1007-1015 (1984); Izant, *et al.*, Science 229:345-352 (1985) and De Benedetti, *et al.*, Proc. Natl. Acad. Sci. 84:658-662 (1987)). Possible mechanisms for the antisense effect are the blockage of translation or

prevention of splicing, both of which have been observed in vitro. Interference with splicing allows the use of intron sequences (Munroe, EMBO. J. 7:2523-2532 (1988) which should be less conserved and therefore result in greater specificity in inhibiting expression of a protein of one species but not its homologue in another species.

Therapeutic gene regulation is accomplished using the "antisense" approach, in which the function of a target gene in a cell or organism is blocked, by transfection of DNA, preferably an oligonucleotide, encoding antisense RNA which acts specifically to inhibit expression of the particular target gene. The sequence of the antisense DNA is designed to result in a full or preferably partial antisense RNA transcript which is substantially complementary to a segment of the gene or mRNA which it is intended to inhibit. The complementarity must be sufficient so that the antisense RNA can hybridize to the target gene (or mRNA) and inhibit the target gene's function, regardless of whether the action is at the level of splicing, transcription or translation. The degree of inhibition, readily discernible by one of ordinary skill in the art without undue experimentation, must be sufficient to inhibit, or render the cell incapable of expressing, the target gene. One of ordinary skill in the art will recognize that the antisense RNA approach is but one of a number of known mechanisms which can be employed to block specific gene expression.

By the term "antisense" is intended an RNA sequence, as well as a DNA sequence coding therefor, which is sufficiently complementary to a particular mRNA molecule for which the antisense RNA is specific to cause molecular hybridization between the antisense RNA and the mRNA such that translation of the mRNA is inhibited. Such hybridization must occur under in vivo conditions, that is, inside the cell. The action of the antisense RNA results in specific inhibition of gene expression in the cell. See Albers, *et al.*, "Molecular Biology Of The Cell", 2nd Ed., Garland Publishing, Inc., New York, NY (1989), in particular, pages 195-196.

The antisense RNA of the present invention may be hybridizable to any of several portions of a target mRNA, including the coding sequence, a 3' or 5' untranslated region, or other intronic sequences. A preferred antisense RNA is that complementary to hAC6 mRNA. As is readily discernible by one of skill in the art, the minimal amount of homology required by the present invention is that sufficient to result in hybridization to

the specific target mRNA and inhibition of its translation or function while not affecting function of other mRNA molecules and the expression of other genes.

Antisense RNA is delivered to a cell by transformation or transfection with a vector into which has been placed DNA encoding the antisense RNA with the appropriate regulatory sequences, including a promoter, to result in expression of the antisense RNA in a host cell.

"Triple helix" or "triplex" approaches involve production of synthetic oligonucleotides which bind to the major groove of a duplex DNA to form a colinear triplex. Such triplex formation can regulate and inhibit cellular growth. See, for example, Hogan, *et al.*, U.S. Patent 5, 176,996; Cohen, *et al.*, Sci. Amer., Dec. 1994, p. 76-82; Helene, Anticancer Drug Design 6:569-584 (1991); Maher III, *et al.*, Antisense Res. Devel. 1:227-281 (Fall 1991); and Crook, *et al.* eds., "Antisense Research and Applications", CRC Press, 1993; all of which are incorporated herein by reference. It is based in part on the discovery that a DNA oligonucleotide can bind by triplex formation to a duplex DNA target in a gene regulatory region, thereby repressing transcription initiation (Cooney, *et. al.* Science 241:456 (1988)). The present invention utilizes methods such as those of Hogan *et al.*, *supra* , to designing oligonucleotides which will bind tightly and specifically to a duplex DNA target comprising part of the hAC6-encoding DNA or a regulatory sequence thereof. Such triplex oligonucleotides can therefore be used as a class of drug molecules to selectively manipulate the expression of this gene.

Thus the present invention is directed to providing to a cell or administering to a subject a synthetic oligonucleotide in sufficient quantity for cellular uptake and binding to a DNA duplex of the target hAC6-coding DNA sequence or a regulatory sequence thereof, such that the oligonucleotide binds to the DNA duplex to form a colinear triplex. This method is used to inhibit expression of the hAC6 enzyme on cells *in vitro* or *in vivo*. Preferably the target sequence is positioned within the DNA domain adjacent to the RNA transcription origin. This method can also be used to inhibit growth of cells which is dependent on expression of this enzyme. The method may also be used to alter the relative amounts or proportions of the hAC6 expressed on cells or tissues by administering such a triplex-forming synthetic oligonucleotide.

The following examples are intended to illustrate but not to limit the invention.

EXAMPLE 1

Construction and Screening of a human heart cDNA library

Whole human heart was used as a source of mRNA. The libraries were purchased from a commercial source, Clontech (Catalog No. HL3026a). The libraries were prepared in a lambda gt10 phage with both oligo-dT and random primers. The primary screening of the lambda gt10 library was carried out with gentle washing (less stringent conditions). Prehybridization and hybridization were carried out at standard conditions. A suitable PCR AC fragment was used as a probe.

The probe was radiolabeled with ^{32}P-dCTP by the random primer labeling method. After hybridization, the blot was washed under increasingly stringent conditions and then radioautographed. A positive clone was obtained.

The next step was to ascertain the full length cDNA sequence from the inserts in the clones. All the positive clones from the human heart library were subcloned into a suitable plasmid. After restriction maps were made, they were further subcloned and sequenced with universal primers or synthesized oligomers. The sequence was performed bidirectionally with Sequenase (Tabor, *et al.*, Proc. Natl. Acad. Sci. USA 84:4767-4771 (1987).

Clone were either used on their own, or sequenced and then used to generate PCR primers which were used to acquire additional clones of interest, by the PCR-based RACE ("rapid amplification of cDNA ends") technique (Frohman, M.A., Methods Enzymol. 218:340-362 (1991)) and human heart mRNA. One clone of particular interest was used as a probe to screen a separate human heart library and several more clones were obtained. Sequencing revealed an open reading frame of 3504 bases reads through to a TGA, a translation termination codon (Figure 1). Thus, the clone(s) encode a protein of 1168 amino acids. The entire coding portion of the cDNA and its deduced amino acid sequence are shown (Figure 1) (SEQ ID NO: 1 and 2, respectively).

One or more fragments from these clones were subcloned into pcDNA3, obtained from Invitrogen. The resulting expression vector, containing the full length cDNA, was given a designation. Samples of this expression vector, inserted into an appropriate *E. coli* strain designated SURE, were deposited with the American Type Culture Collection,

12301 Parklawn Drive, Rockville, Md. 20852, on _____, 1997 in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and have been accorded accession number ATCC _____.

EXAMPLE 2

Cloning and Expression of the human type VI adenylyl cyclase

The human type VI adenylyl cyclase was produced by cloning and expressing heart type VI adenylyl cyclase cDNA in a suitable expression system using recombinant DNA methods, such as are well known in the art.

Purified plasmid was transfected into HEK-293 cells using electroporation. The cells were grown in an appropriate growth medium then washed. After the addition of trypsin solution, the cells were incubated, harvested and resuspended in the growth media. Purified plasmid was added to an electroporation cuvette. Cells were added to the DNA and the mixture was pulsed. The cell-DNA mixture was then plated into a suitable growth media. The plate was incubated before placing cells on a suitable selective media.

hAC6, having 1168 amino acids, was analyzed for secondary structure by the method of Kyte, *et al.*, J. Mol. Biol. 157:105-132 (1982). The software, GeneWorks; v.2.45; IntelliGenetics, Inc. ; Mountain View; California was used to obtain a hydropathy plot, and thereby identify the membrane related structure of this adenylyl cyclase isoform. The method of Kyte, *et al.*, *supra*, was used with a window size of 5.

Twelve peaks appear in the hydropathy plot, not shown, which represent transmembrane spanning regions. These results suggest that this adenylyl cyclase isoform has a structure of twelve transmembrane spanning regions, as well as a large cytoplasmic loop located in the middle and at the end, which is consistent with the structures of the previously characterized isoforms.

EXAMPLE 3

Evaluation of the human type VI adenylyl cyclase

The biochemical characteristics of hAC9 were determined in a stable expression system using HEK-293 cells. A fragment of the adenylyl cyclase cDNA containing the whole coding sequence was inserted into a suitable plasmid.

An assay was performed to measure cAMP product formation and it was determined that the hAC6 enzyme expressed by this cDNA was active.

EXAMPLE 4

Tissue distribution of the human type VI adenylyl cyclase

In order to determine the tissue distribution of hAC6, Northern blotting was performed using mRNA from various tissues. Messenger RNA was purified using guanidium sodium and oligo-dT columns from various human tissues.

The blot was pre-hybridized in a suitable solution before the addition of a probe. Hybridization was performed, followed by washing under increasingly stringent conditions. The blot was then autoradiographed.

The results of the Northern blot analysis indicated that hAC6 is predominantly expressed in heart and brain tissue, although some expression was also found at slight levels in other tissues.

All references cited and mentioned above, including patents, journal articles and texts, are all incorporated by reference herein, whether expressly incorporated or not.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

Claims

What is claimed is:

1. An isolated and purified human type VI adenylyl cyclase polypeptide comprising the amino acid sequence of Figure 1 (SEQ ID NO:2).

2. An isolated and purified nucleic acid encoding for human type VI adenylyl cyclase.

3. An isolated and purified nucleic acid comprising the nucleotide sequence of Figure 1 (SEQ ID NO:1), which encodes a biologically active human type VI adenylyl cyclase.

4. The nucleic acid of Claim 3, which encodes a biologically active soluble human type VI adenylyl cyclase peptide fragment.

5. A method of producing human type VI adenylyl cyclase which comprises incorporating the nucleic acid of claim 2 into an expression vector, transforming a host cell with said vector and culturing the transformed host cell under conditions which result in expression of the gene.

6. The method of claim 5 wherein the host cell comprises a bacterial, viral, yeast, insect or mammalian cell line.

7. The method of Claim 6 wherein said host cells are HEK-293 cells.

FIG. 1A


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GCGGACGGCG GACGGCGGAC GGCGGGCGGG ACGGCCAGGA CGCGCGGGCT    50
CGCCTGCCGC CTGCCGCCTG CCGCCCGCCC TGCCGGTCCT GCGCGCCCGA

CGGAGGACCG CGGGACGGCC GGCCGGCCGG CCGGAGCCCG CGGGGCGGC   100
GCCTCCTGGC GCCCTGCCGG CCGGCCGGCC GGCCTCGGGC GCCCCCGCCG

GGGGCGGGGG CCCAGGGCAG GCGCGGAGCC GGGCCGGCAG CAACATGTCA  150
CCCCGCCCCC GGGTCCCGTC CGCGCCTCGG CCCGGCCGTC GTTGTACAGT
                                                  MetSer

TGGTTTAGTG GCCTCCTGGT CCCTAAAGTG GATGAACGGA AAACAGCCTG  200
ACCAAATCAC CGGAGGACCA GGGATTTCAC CTACTTGCCT TTTGTCGGAC
TrpPheSerG lyLeuLeuVa lProLysVal AspGluArgL ysThrAlaTr

GGGTGAACGC AATGGGCAGA AGCGTTCGCG GCGCCGTGGC ACTCGGGCAG  250
CCCACTTGCG TTACCCGTCT TCGCAAGCGC CGCGGCACCG TGAGCCCGTC
pGlyGluArg AsnGlyGlnL ysArgSerAr gArgArgGly ThrArgAlaG

GTGGCTTCTG CACGCCCCGC TATATGAGCT GCCTCCGGGA TGCAGAGCCA  300
CACCGAAGAC GTGCGGGGCG ATATACTCGA CGGAGGCCCT ACGTCTCGGT
lyGlyPheCy sThrProArg TyrMetSerC ysLeuArgAs pAlaGluPro

CCCAGCCCCA CCCCTGCGGG CCCCCCTCGG TGCCCCTGGC AGGATGACGC  350
GGGTCGGGGT GGGGACGCCC GGGGGGAGCC ACGGGGACCG TCCTACTGCG
ProSerProT hrProAlaGl yProProArg CysProTrpG lnAspAspAl

CTTCATCCGG AGGGGCGGCC CAGGCAAGGG CAAGGAGCTG GGGCTGCGGG  400
GAAGTAGGCC TCCCCGCCGG GTCCGTTCCC GTTCCTCGAC CCCGACGCCC
aPheIleArg ArgGlyGlyP roGlyLysGl yLysGluLeu GlyLeuArgA

CAGTGGCCCT GGGCTTCGAG GATACCGAGG TGACAACGAC AGCGGGCGGG  450
GTCACCGGGA CCCGAAGCTC CTATGGCTCC ACTGTTGCTG TCGCCCGCCC
laValAlaLe uGlyPheGlu AspThrGluV alThrThrTh rAlaGlyGly

ACGGCTGAGG TGGCGCCCGA CGCGGTGCCC AGGAGTGGGA GATCCTGCTG  500
TGCCGACTCC ACCGCGGGCT GCGCCACGGG TCCTCACCCT CTAGGACGAC
ThrAlaGluV alAlaProAs pAlaValPro ArgSerGlyA rgSerCysTr

GCGCCGTCTG GTGCAGGTGT TCCAGTCGAA GCAGTTCCGT TCGGCCAAGC  550
CGCGGCAGAC CACGTCCACA AGGTCAGCTT CGTCAAGGCA AGCCGGTTCG
pArgArgLeu ValGlnValP heGlnSerLy sGlnPheArg SerAlaLysL

TGGAGCACCT GTACCAGCGG TACTTCTTCC AGATGAACCA GAGCAGCCTG  600
ACCTCGTGGA CATGGTCGCC ATGAAGAAGG TCTACTTGGT CTCGTCGGAC
euGluHisLe uTyrGlnArg TyrPhePheG lnMetAsnGl nSerSerLeu
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FIG. 1B


```
ACGCTGCTGA TGGCGGTGCT GGTGCTGCTC ACAGCGGTGC TGCTGGCTTT   650
TGCGACGACT ACCGCCACGA CCACGACGAG TGTCGCCACG ACGACCGAAA
ThrLeuLeuM etAlaValLe uValLeuLeu ThrAlaValL euLeuAlaPh

CCACGCCGCA CCCGCCCGCC CTCAGCCTGC CTATGTGGCA CTGTTGGCCT   700
GGTGCGGCGT GGGCGGGCGG GAGTCGGACG GATACACCGT GACAACCGGA
eHisAlaAla ProAlaArgP roGlnProAl aTyrValAla LeuLeuAlaC

GTGCCGCCGC CCTGTTCGTG GGGCTCATGG TGGTGTGTAA CCGGCATAGC   750
CACGGCGGCG GGACAAGCAC CCCGAGTACC ACCACACATT GGCCGTATCG
ysAlaAlaAl aLeuPheVal GlyLeuMetV alValCysAs nArgHisSer

TTCCGCCAGG ACTCCATGTG GGTGGTGAGC TACGTGGTGC TGGGCATCCT   800
AAGGCGGTCC TGAGGTACAC CCACCACTCG ATGCACCACG ACCCGTAGGA
PheArgGlnA spSerMetTr pValValSer TyrValValL euGlyIleLe

GGCGGCAGTG CAGGTCGGGG GCGCTCTCGC AGCAGACCCG CGCAGCCCCT   850
CCGCCGTCAC GTCCAGCCCC CGCGAGAGCG TCGTCTGGGC GCGTCGGGGA
uAlaAlaVal GlnValGlyG lyAlaLeuAl aAlaAspPro ArgSerProS

CTGCGGGCCT CTGGTGCCCT GTGTTCTTTG TCTACATCGC CTACACGCTC   900
GACGCCCGGA GACCACGGGA CACAAGAAAC AGATGTAGCG GATGTGCGAG
erAlaGlyLe uTrpCysPro ValPhePheV alTyrIleAl aTyrThrLeu

CTCCCCATCC GCATGCGGGC TGCCGTCCTC AGCGGCCTGG GCCTCTCCAC   950
GAGGGGTAGG CGTACGCCCG ACGGCAGGAG TCGCCGGACC CGGAGAGGTG
LeuProIleA rgMetArgAl aAlaValLeu SerGlyLeuG lyLeuSerTh

CTTGCATTTG ATCTTGGCCT GGCAACTTAA CCGTGGTGAT GCCTTCCTCT  1000
GAACGTAAAC TAGAACCGGA CCGTTGAATT GGCACCACTA CGGAAGGAGA
rLeuHisLeu IleLeuAlaT rpGlnLeuAs nArgGlyAsp AlaPheLeuT

GGAAGCAGCT CGGTGCCAAT GTGCTGCTGT TCCTCTGCAC CAACGTCATT  1050
CCTTCGTCGA GCCACGGTTA CACGACGACA AGGAGACGTG GTTGCAGTAA
rpLysGlnLe uGlyAlaAsn ValLeuLeuP heLeuCysTh rAsnValIle

GGCATCTGCA CACACTATCC AGCAGAGGTG TCTCAGCGCC AGGCCTTTCA  1100
CCGTAGACGT GTGTGATAGG TCGTCTCCAC AGAGTCGCGG TCCGGAAAGT
GlyIleCysT hrHisTyrPr oAlaGluVal SerGlnArgG lnAlaPheGl

GGAGACCCGC GGTTACATCC AGGCCCGGCT CCACCTGCAG CATGAGAATC  1150
CCTCTGGGCG CCAATGTAGG TCCGGGCCGA GGTGGACGTC GTACTCTTAG
nGluThrArg GlyTyrIleG lnAlaArgLe uHisLeuGln HisGluAsnA

GGCAGCAGGA GCGGCTGCTG CTGTCGGTAT TGCCCCAGCA CGTTGCCATG  1200
CCGTCGTCCT CGCCGACGAC GACAGCCATA ACGGGGTCGT GCAACGGTAC
rgGlnGlnGl uArgLeuLeu LeuSerValL euProGlnHi sValAlaMet
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FIG. IC

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GAGATGAAAG AAGACATCAA CACAAAAAAA GAAGACATGA TGTTCCACAA  1250
CTCTACTTTC TTCTGTAGTT GTGTTTTTTT CTTCTGTACT ACAAGGTGTT
GluMetLysG luAspIleAs nThrLysLys GluAspMetM etPheHisLy

GATCTACATA CAGAAGCATG ACAATGTCAG CATCCTGTTT GCAGACATTG  1300
CTAGATGTAT GTCTTCGTAC TGTTACAGTC GTAGGACAAA CGTCTGTAAC
sIleTyrIle GlnLysHisA spAsnValSe rIleLeuPhe AlaAspIleG

AGGGCTTCAC CAGCCTGGCA TCCCAGTGCA CTGCGCAGGA GCTGGTCATG  1350
TCCCGAAGTG GTCGGACCGT AGGGTCACGT GACGCGTCCT CGACCAGTAC
luGlyPheTh rSerLeuAla SerGlnCysT hrAlaGlnGl uLeuValMet

ACCCTGAATG AGCTCTTTGC CCGGTTTGAC AAGCTGGCTG CGGAGAATCA  1400
TGGGACTTAC TCGAGAAACG GGCCAAACTG TTCGACCGAC GCCTCTTAGT
ThrLeuAsnG luLeuPheAl aArgPheAsp LysLeuAlaA laGluAsnHi

CTGCCTGAGG ATCAAGATCT TGGGGGACTG TTACTACTGT GTGTCAGGGC  1450
GACGGACTCC TAGTTCTAGA ACCCCCTGAC AATGATGACA CACAGTCCCG
sCysLeuArg IleLysIleL euGlyAspCy sTyrTyrCys ValSerGlyL

TGCCGGAGGC CCGGGCCGAC CATGCCCACT GCTGTGTGGA GATGGGGGTA  1500
ACGGCCTCCG GGCCCGGCTG GTACGGGTGA CGACACACCT CTACCCCCAT
euProGluAl aArgAlaAsp HisAlaHisC ysCysValGl uMetGlyVal

GACATGATTG AGGCCATCTC GCTGGTACGT GAGGTGACAG GTGTGAATGT  1550
CTGTACTAAC TCCGGTAGAG CGACCATGCA CTCCACTGTC CACACTTACA
AspMetIleG luAlaIleSe rLeuValArg GluValThrG lyValAsnVa

GAACATGCGC GTGGGCATCC ACAGCGGGCG CGTGCACTGC GGCGTCCTTG  1600
CTTGTACGCG CACCCGTAGG TGTCGCCCGC GCACGTGACG CCGCAGGAAC
lAsnMetArg ValGlyIleH isSerGlyAr gValHisCys GlyValLeuG

GCTTGCGGAA ATGGCAGTTC GATGTGTGGT CCAATGATGT GACCCTGGCC  1650
CGAACGCCTT TACCGTCAAG CTACACACCA GGTTACTACA CTGGGACCGG
lyLeuArgLy sTrpGlnPhe AspValTrpS erAsnAspVa lThrLeuAla

AACCACATGG AGGCAGGAGG CCGGGCTGGC CGCATCCACA TCACTCGGGC  1700
TTGGTGTACC TCCGTCCTCC GGCCCGACCG GCGTAGGTGT AGTGAGCCCG
AsnHisMetG luAlaGlyGl yArgAlaGly ArgIleHisI leThrArgAl

AACACTGCAG TACCTGAACG GGGACTACGA GGTGGAGCCA GGCCGTGGTG  1750
TTGTGACGTC ATGGACTTGC CCCTGATGCT CCACCTCGGT CCGGCACCAC
aThrLeuGln TyrLeuAsnG lyAspTyrGl uValGluPro GlyArgGlyG

GCGAGCGCAA CGCGTACCTC AAGGAGCAGC ACATTGAGAC TTTCCTCATC  1800
CGCTCGCGTT GCGCATGGAG TTCCTCGTCG TGTAACTCTG AAAGGAGTAG
lyGluArgAs nAlaTyrLeu LysGluGlnH isIleGluTh rPheLeuIle
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FIG. 1D


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CTGGGCGCCA GCCAGAAACG GAAAGAGGAG AAGGCCATGC TGGCCAAGCT 1850
GACCCGCGGT CGGTCTTTGC CTTTCTCCTC TTCCGGTACG ACCGGTTCGA
LeuGlyAlaS erGlnLysAr gLysGluGlu LysAlaMetL euAlaLysLe

GCAGCGGACT CGGGCCAACT CCATGGAAGG GCTGATGCCG CGCTGGGTTC 1900
CGTCGCCTGA GCCCGGTTGA GGTACCTTCC CGACTACGGC GCGACCCAAG
uGlnArgThr ArgAlaAsnS erMetGluGl yLeuMetPro ArgTrpValP

CTGATCGTGC CTTCTCCCGG ACCAAGGACT CCAAGGCCTT CCGCCAGATG 1950
GACTAGCACG GAAGAGGGCC TGGTTCCTGA GGTTCCGGAA GGCGGTCTAC
roAspArgAl aPheSerArg ThrLysAspS erLysAlaPh eArgGlnMet

GGCATTGATG ATTCCAGCAA AGACAACCGG GGCACCCAAG ATGCCCTGAA 2000
CCGTAACTAC TAAGGTCGTT TCTGTTGGCC CCGTGGGTTC TACGGGACTT
GlyIleAspA spSerSerLy sAspAsnArg GlyThrGlnA spAlaLeuAs

CCCTGAGGAT GAGGTGGATG AGTTCCTGAG CCGTGCCATC GATGCCCGCA 2050
GGGACTCCTA CTCCACCTAC TCAAGGACTC GGCACGGTAG CTACGGGCGT
nProGluAsp GluValAspG luPheLeuSe rArgAlaIle AspAlaArgS

GCATTGATCA GCTGCGGAAG GACCATGTGC GCCGGTTTCT GCTCACCTTC 2100
CGTAACTAGT CGACGCCTTC CTGGTACACG CGGCCAAAGA CGAGTGGAAG
erIleAspGl nLeuArgLys AspHisValA rgArgPheLe uLeuThrPhe

CAGAGAGAGG ATCTTGAGAA GAAGTACTCC CGGAAGGTGG ATCCCCGCTT 2150
GTCTCTCTCC TAGAACTCTT CTTCATGAGG GCCTTCCACC TAGGGGCGAA
GlnArgGluA spLeuGluLy sLysTyrSer ArgLysValA spProArgPh

CGGAGCCTAC GTTGCCTGTG CCCTGTTGGT CTTCTGCTTC ATCTGCTTCA 2200
GCCTCGGATG CAACGGACAC GGGACAACCA GAAGACGAAG TAGACGAAGT
eGlyAlaTyr ValAlaCysA laLeuLeuVa lPheCysPhe IleCysPheI

TCCAGCTTCT CATCTTCCCA CACTCCACCC TGATGCTTGG GATCTATGCC 2250
AGGTCGAAGA GTAGAAGGGT GTGAGGTGGG ACTACGAACC CTAGATACGG
leGlnLeuLe uIlePhePro HisSerThrL euMetLeuGl yIleTyrAla

AGCATCTTCC TGCTGCTGCT AATCACCGTG CTGATCTGTG CTGTGTACTC 2300
TCGTAGAAGG ACGACGACGA TTAGTGGCAC GACTAGACAC GACACATGAG
SerIlePheL euLeuLeuLe uIleThrVal LeuIleCysA laValTyrSe

CTGTGGTTCT CTGTTCCCTA AGGCCCTGCA ACGTCTGTCC CGCAGCATTG 2350
GACACCAAGA GACAAGGGAT TCCGGGACGT TGCAGACAGG GCGTCGTAAC
rCysGlySer LeuPheProL ysAlaLeuGl nArgLeuSer ArgSerIleV

TCCGCTCACG GGCACATAGC ACCGCAGTTG GCATCTTTTC CGTCCTGCTT 2400
AGGCGAGTGC CCGTGTATCG TGGCGTCAAC CGTAGAAAAG GCAGGACGAA
alArgSerAr gAlaHisSer ThrAlaValG lyIlePheSe rValLeuLeu
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FIG. 1E


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GTGTTTACTT CTGCCATTGC CAACATGTTC ACCTGTAACC ACACCCCCAT 2450
CACAAATGAA GACGGTAACG GTTGTACAAG TGGACATTGG TGTGGGGGTA
ValPheThrS erAlaIleAl aAsnMetPhe ThrCysAsnH isThrProIl

ACGGAGCTGT GCAGCCCGGA TGCTGAATTT AACACCTGCT GACATCACTG 2500
TGCCTCGACA CGTCGGGCCT ACGACTTAAA TTGTGGACGA CTGTAGTGAC
eArgSerCys AlaAlaArgM etLeuAsnLe uThrProAla AspIleThrA

CCTGCCACCT GCAGCAGCTC AATTACTCTC TGGGCCTGGA TGCTCCCCTG 2550
GGACGGTGGA CGTCGTCGAG TTAATGAGAG ACCCGGACCT ACGAGGGGAC
laCysHisLe uGlnGlnLeu AsnTyrSerL euGlyLeuAs pAlaProLeu

TGTGAGGGCA CCATGCCCAC CTGCAGCTTT CCTGAGTACT TCATCGGGAA 2600
ACACTCCCGT GGTACGGGTG GACGTCGAAA GGACTCATGA AGTAGCCCTT
CysGluGlyT hrMetProTh rCysSerPhe ProGluTyrP heIleGlyAs

CATGCTGCTG AGTCTCTTGG CCAGCTCTGT CTTCCTGCAC ATCAGCAGCA 2650
GTACGACGAC TCAGAGAACC GGTCGAGACA GAAGGACGTG TAGTCGTCGT
nMetLeuLeu SerLeuLeuA laSerSerVa lPheLeuHis IleSerSerI

TCGGGAAGTT GGCCATGATC TTTGTCTTGG GGCTCATCTA TTTGGTGCTG 2700
AGCCCTTCAA CCGGTACTAG AAACAGAACC CCGAGTAGAT AAACCACGAC
leGlyLysLe uAlaMetIle PheValLeuG lyLeuIleTy rLeuValLeu

CTTCTGCTGG GTCCCCCAGC CACCATCTTT GACAACTATG ACCTACTGCT 2750
GAAGACGACC CAGGGGGTCG GTGGTAGAAA CTGTTGATAC TGGATGACGA
LeuLeuLeuG lyProProAl aThrIlePhe AspAsnTyrA spLeuLeuLe

TGGCGTCCAT GGCTTGGCTT CTTCCAATGA GACCTTTGAT GGGCTGGACT 2800
ACCGCAGGTA CCGAACCGAA GAAGGTTACT CTGGAAACTA CCCGACCTGA
uGlyValHis GlyLeuAlaS erSerAsnGl uThrPheAsp GlyLeuAspC

GTCCAGCTGC AGGGAGGGTG GCCCTCAAAT ATATGACCCC TGTGATTCTG 2850
CAGGTCGACG TCCCTCCCAC CGGGAGTTTA TATACTGGGG ACACTAAGAC
ysProAlaAl aGlyArgVal AlaLeuLysT yrMetThrPr oValIleLeu

CTGGTGTTTG CGCTGGCGCT GTATCTGCAT GCTCAGCAGG TGGAGTCGAC 2900
GACCACAAAC GCGACCGCGA CATAGACGTA CGAGTCGTCC ACCTCAGCTG
LeuValPheA laLeuAlaLe uTyrLeuHis AlaGlnGlnV alGluSerTh

TGCCCGCCTA GACTTCCTCT GGAAACTACA GGCAACAGGG GAGAAGGAGG 2950
ACGGGCGGAT CTGAAGGAGA CCTTTGATGT CCGTTGTCCC CTCTTCCTCC
rAlaArgLeu AspPheLeuT rpLysLeuGl nAlaThrGly GluLysGluG

AGATGGAGGA GCTACAGGCA TACAACCGGA GGCTGCTGCA TAACATTCTG 3000
TCTACCTCCT CGATGTCCGT ATGTTGGCCT CCGACGACGT ATTGTAAGAC
luMetGluGl uLeuGlnAla TyrAsnArgA rgLeuLeuHi sAsnIleLeu
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FIG. 1F


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CCCAAGGACG TGGCGGCCCA CTTCCTGGCC CGGGAGCGCC GCAATGATGA 3050
GGGTTCCTGC ACCGCCGGGT GAAGGACCGG GCCCTCGCGG CGTTACTACT
ProLysAspV alAlaAlaHi sPheLeuAla ArgGluArgA rgAsnAspGl

ACTCTACTAT CAGTCGTGTG AGTGTGTGGC TGTTATGTTT GCCTCCATTG 3100
TGAGATGATA GTCAGCACAC TCACACACCG ACAATACAAA CGGAGGTAAC
uLeuTyrTyr GlnSerCysG luCysValAl aValMetPhe AlaSerIleA

CCAACTTCTC TGAGTTCTAT GTGGAGCTGG AGGCAAACAA TGAGGGTGTC 3150
GGTTGAAGAG ACTCAAGATA CACCTCGACC TCCGTTTGTT ACTCCCACAG
laAsnPheSe rGluPheTyr ValGluLeuG luAlaAsnAs nGluGlyVal

GAGTGCCTGC GGCTGCTCAA CGAGATCATC GCTGACTTTG ATGAGATTAT 3200
CTCACGGACG CCGACGAGTT GCTCTAGTAG CGACTGAAAC TACTCTAATA
GluCysLeuA rgLeuLeuAs nGluIleIle AlaAspPheA spGluIleIl

CAGCGAGGAG CGGTTCCGGC AGCTGGAAAA GATCAAGACG ATTGGTAGCA 3250
GTCGCTCCTC GCCAAGGCCG TCGACCTTTT CTAGTTCTGC TAACCATCGT
eSerGluGlu ArgPheArgG lnLeuGluLy sIleLysThr IleGlySerT

CCTACATGGC TGCCTCAGGG CTGAACGCCA GCACCTACGA TCAGGTGGGC 3300
GGATGTACCG ACGGAGTCCC GACTTGCGGT CGTGGATGCT AGTCCACCCG
nrTyrMetAl aAlaSerGly LeuAsnAlaS erThrTyrAs pGlnValGly

CGCTCCCACA TCACTGCCCT GGCTGACTAC GCCATGCGGC TCATGGAGCA 3350
GCGAGGGTGT AGTGACGGGA CCGACTGATG CGGTACGCCG AGTACCTCGT
ArgSerHisI leThrAlaLe uAlaAspTyr AlaMetArgL euMetGluGl

GATGAAGCAC ATCAATGAGC ACTCCTTCAA CAATTTCCAG ATGAAGATTG 3400
CTACTTCGTG TAGTTACTCG TGAGGAAGTT GTTAAAGGTC TACTTCTAAC
nMetLysHis IleAsnGluH isSerPheAs nAsnPheGln MetLysIleG

GGCTGAACAT GGGCCCAGTC GTGGCAGGTG TCATCGGGGC TCGGAAGCCA 3450
CCGACTTGTA CCCGGGTCAG CACCGTCCAC AGTAGCCCCG AGCCTTCGGT
lyLeuAsnMe tGlyProVal ValAlaGlyV alIleGlyAl aArgLysPro

CAGTATGACA TCTGGGGGAA CACAGTGAAT GTCTCTAGTC GTATGGACAG 3500
GTCATACTGT AGACCCCCTT GTGTCACTTA CAGAGATCAG CATACCTGTC
GlnTyrAspI leTrpGlyAs nThrValAsn ValSerSerA rgMetAspSe

CACGGGGGTC CCCGACCGAA TCCAGGTGAC CACGGACCTG TACCAGGTTC 3550
GTGCCCCCAG GGGCTGGCTT AGGTCCACTG GTGCCTGGAC ATGGTCCAAG
rThrGlyVal ProAspArgI leGlnValTh rThrAspLeu TyrGlnValL

TAGCTGCCAA GGGCTACCAG CTGGAGTGTC GAGGGGTGGT CAAGGTGAAG 3600
ATCGACGGTT CCCGATGGTC GACCTCACAG CTCCCCACCA GTTCCACTTC
euAlaAlaLy sGlyTyrGln LeuGluCysA rgGlyValVa lLysValLys
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FIG. 1G

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GGCAAGGGGG AGATGACCAC CTACTTCCTC AATGGGGGCC CCAGCAGTTA  3650
CCGTTCCCCC TCTACTGGTG GATGAAGGAG TTACCCCCGG GGTCGTCAAT
GlyLysGlyG luMetThrTh rTyrPheLeu AsnGlyGlyP roSerSer

ACAGGGCCCA GCCACAAATT CAGCTGAAGG GACCAAGGTG GGCATTGAGT  3700
TGTCCCGGGT CGGTGTTTAA GTCGACTTCC CTGGTTCCAC CCGTAACTCA

GGACTCTGTG CTCACTGGGT GGAGCTGTGG CAGGGGGCAC TGAGCCTCCA  3750
CCTGAGACAC GAGTGACCCA CCTCGACACC GTCCCCCGTG ACTCGGAGGT

GACCCTGCTA ACCACAAAAG GGAACATCCC AGCAGGCTGT GCTTGGATCA  3800
CTGGGACGAT TGGTGTTTTC CCTTGTAGGG TCGTCCGACA CGAACCTAGT

TGCTCGTCTG CCCTCAAGCT GGAAAACAAG GGGCTACCTA CCGAGAGGAT  3850
ACGAGCAGAC GGGAGTTCGA CCTTTTGTTC CCCGATGGAT GGCTCTCCTA

TATGCAAGTG ACTTTCTTTC TTACTTGGGG TAGGGCTGTT CCCTCTCCAA  3900
ATACGTTCAC TGAAAGAAAG AATGAACCCC ATCCCGACAA GGGAGAGGTT

TCTTCCAGCC TTTGGGAGCA GGGGAGGGGT CAGTAGCAGA AGCAGAGGGA  3950
AGAAGGTCGG AAACCCTCGT CCCCTCCCCA GTCATCGTCT TCGTCTCCCT

GGCCTCTTGC CTGAGGGATT AAAATGGCAG CTTGCCATGC CTACCCTTCC  4000
CCGGAGAACG GACTCCCTAA TTTTACCGTC GAACGGTACG GATGGGAAGG

CTGTCTGTCT GGGCAGCAGG TTCAGGGCTG AGCCCTTCTT TTCCCTCTTT  4050
GACAGACAGA CCCGTCGTCC AAGTCCCGAC TCGGGAAGAA AAGGGAGAAA

TTTCCTGGGA ATATTTTGTA CAATATTTTG TACAAAGACA GGCATGAGGA  4100
AAAGGACCCT TATAAAACAT GTTATAAAAC ATGTTTCTGT CCGTACTCCT

GTGCCTATTC CATGCTTGCC TTTGCAATAC CTGCATCCCC AGCACTGGTC  4150
CACGGATAAG GTACGAACGG AAACGTTATG GACGTAGGGG TCGTGACCAG

CTGGGCACTT CCCCACCCCA GCCAGGTGTC CCTCCTATGC ACAGAGCAGA  4200
GACCCGTGAA GGGGTGGGGT CGGTCCACAG GGAGGATACG TGTCTCGTCT
```

FIG. 1H

GGAGGGAGAA GCTCTGGGGA GCCAGCTTTG GCCATATTTC AGGAGAATGT 4250
CCTCCCTCTT CGAGACCCCT CGGTCGAAAC CGGTATAAAG TCCTCTTACA

TTCCATGTGC CAAATCTTAG TCCCATGATC TGTCCCCAAA GGGGAACAAA 4300
AAGGTACACG GTTTAGAATC AGGGTACTAG ACAGGGGTTT CCCCTTGTTT

GGGACCTCTG ACAGCTTAGA TTTAGCCCCA GTTCCTGCAC GCTCCAGGGA 4350
CCCTGGAGAC TGTCGAATCT AAATCGGGGT CAAGGACGTG CGAGGTCCCT

ACGGGGTGTC TGGCCTCACT GGTACTGTGA AAAATGCTCA GAGAGCAAGC 4400
TGCCCCACAG ACCGGAGTGA CCATGACACT TTTTACGAGT CTCTCGTTCG

CTGTGTGTGG GGATGTCAGG TCAGGAGCTG GAAGTTCACC TGCAGGTGCC 4450
GACACACACC CCTACAGTCC AGTCCTCGAC CTTCAAGTGG ACGTCCACGG

AAAGAGCAGG CCGGCCAGGG CTGGGGCAGT GCCAGACTCT GATCTGAGGA 4500
TTTCTCGTCC GGCCGGTCCC GACCCCGTCA CGGTCTGAGA CTAGACTCCT

CCCCGTCGGG GTCCAGATCA GGTCACTCTG CCCCAGTGCT CTCTTGCTGT 4550
GGGGCAGCCC CAGGTCTAGT CCAGTGAGAC GGGGTCACGA GAGAACGACA

CTGCTGACAA GGGGGCATGG AGCATCTCTT CCTCTTCTGT TGCCAAATAG 4600
GACGACTGTT CCCCCGTACC TCGTAGAGAA GGAGAAGACA ACGGTTTATC

AAAAGGGTCA GGGCATGGAG AAAGGTGACC CTGATCCCAA ACCTGCCCTC 4650
TTTTCCCAGT CCCGTACCTC TTTCCACTGG GACTAGGGTT TGGACGGGAG

CCAAGTCTCT GGTGTTGGGG AGGGCCCGTG TGTTTGTGTA ACTGTGTGTG 4700
GGTTCAGAGA CCACAACCCC TCCCGGGCAC ACAAACACAT TGACACACAC

CATGTTGGTC TTTGTGTGCA TATCTGTTTT CCAGGTCTAT GTGAGTCCTT 4750
GTACAACCAG AAACACACGT ATAGACAAAA GGTCCAGATA CACTCAGGAA

GTGCTCCTGC TCCTCAGCTC TCCACCCCAG GTTGCCTCTC TCCTGTGGGC 4800
CACGAGGACG AGGAGTCGAG AGGTGGGGTC CAACGGAGAG AGGACACCCG

FIG. 11

CTCTGTCTTC TGGGAATAAA GCAGGGTTTC CTACTTCAGG GGATGTAGAG 4850
GAGACAGAAG ACCCTTATTT CGTCCCAAAG GATGAAGTCC CCTACATCTC

AGATGCCCAG GTTGCACAGG AGTGGGATGG GGTGTGGTAG CAAAAGGAGG 4900
TCTACGGGTC CAACGTGTCC TCACCCTACC CCACACCATC GTTTTCCTCC

GAGAGGAGTC CTTTTTGTGC CAAATCCCTA AGTGCCGTTC GG 4942
CTCTCCTCAG GAAAAACACG GTTTAGGGAT TCACGGCAAG CC

INTERNATIONAL SEARCH REPORT

Inte .ional Application No
PCT/US 98/13694

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/88 C12N15/60 C12N15/85 C12N5/10

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HELLEVUO K ET AL: "A novel adenylyl cyclase sequence cloned from the human erythroleukemia cell line." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1993 APR 15) 192 (1) 311-8. JOURNAL CODE: 9Y8. ISSN: 0006-291X., XP002084072 * see esp. p.316 last par.; figure 1 * ---	1-7
A	HABER N. ET AL.: "Chromosomal mapping of human adenylyl cyclase genes type III, type V and type VI" HUM. GENET. (1994), 94(1), 69-73 , XP002084073 see the whole document ---	1-7

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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 November 1998	27/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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Int. tional Application No
PCT/US 98/13694

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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